Research Paper :

Morphological variation in *Sclerotium rolfsii* Sacc. isolates causing stem rot in groundnut (*Arachis hypogaea* L.)

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SUMMARY

Variability among 59 isolates of *S. rolfsii* collected from groundnut growing areas of Gujarat and Maharashtra states of India was studied. Besides morphological characters (growth rate, colony type, number, size and colour of sclerotia) vegetative compatibility and virulence of the isolates were considered for study of variability. The results revealed that out of 59 isolates, colonies of 35 isolates were fluffy, whereas 24 were compact. Twenty nine isolates were fast growing (diam. >80-90 mm), 24 moderately fast growing (61-79 mm) and 6 were slow growing (<60 mm). Twelve isolates produced a large number of sclerotia (>500 sclerotia/plate) but smaller in size (0.5-1.4), while 9 isolates produced relatively fewer sclerotia (140-286 sclerotia/plate) but larger in size (2.1-2.5 mm). The colour of sclerotia was dark brown, reddish brown and light brown. Nine isolates were highly virulent causing more than 60% mortality of plants due to stem rot. Vegetative compatibility matrix revealed that out of 3481 combinations only 1512 were compatible (44%). On the basis of compatibility and virulence four, isolates *viz*., NRCG-SR 6, 7, 18 and 57 were identified that could be used in a consortium for development of sick plot.

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Stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. has become a major constraint and potential threat to groundnut production in many warm humid areas of the world. Stem rot is also known as wilt, sclerotial disease, blight, foot rot, white mold, southern stem rot southern blight, sclerotium rot (Aycock, 1966, Kokalis-Burelles *et al.*, 1997), southern wilt, southern stem rot, Sclerotial wilt (Patil and Rane, 1983). The yield losses of over 25% have been reported by Mayee and Datar (1988) in Maharashtra. In India, stem rot occurs in all groundnut growing states, particularly it is most severe in Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Andhra Pradesh, Orissa and Tamil Nadu, where it is estimated that over 50,000 ha of groundnut fields are infected with *S. rolfsii* pathogen (Mehan *et al.*, 1995).

S. rolfsii Sacc. is a devastating soil-borne plant pathogenic fungus with a wide host range (Aycock, 1966; Punja, 1988). Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Harlton *et al.,* 1995; Nalim *et al.,* 1995; Okabe *et al.,* 1998). Studies on variability within the population in a geographical region are important because that document the changes occurring in the population. The importance could be realized in the light of the discovery of PCNB-tolerant strains of *S. rolfsii* isolated from a Texas peanut field in 1985 (Nelin, 1992). *S. rolfsii* has prolific growth and ability to produce persistent sclerotia contributing to a higher degree of economic loss (Mahen *et al.,* 1995).

Hence, the present study was carried at the variability in morphology, duration of sclerotia formation, mycelial growth rate, size, colour, number of sclerotia and compatibility of 59 isolates of *S. rolfsii* obtained from groundnut infected plants, and collected at hot spot location of Maharashtra and Gujarat state.

MATERIALS AND METHODS

Isolation and maintenance of *S. rolfsii* isolates:

Fifty nine isolates of *S. rolfsii* causing stem rot of groundnut were collected from

infected groundnut plants and soil samples. The diseased groundnut plants and soil samples were collected from groundnut growing pockets of Maharashtra and Gujarat states during *Kharif* 2008. Collected groundnut plants and soil samples were brought to the laboratory in separate polythene bags.

Isolation from infected plants:

As soon as the infected groundnut plants were brought to laboratory, small bits of infected stem were removed carefully and surface sterilized with 0.1% $Hgcl_2$ and were transferred to Petridishes containing Potato dextrose agar medium (PDA). These plates were incubated for 5 days at 28^0 C in an incubator. After 5 days of incubation to get the pure culture sub-culturing was done from individual samples. This culture was then incubated for 20 days for sclerotia formation. The isolates were further purified by growing single sclerotium on PDA. These isolates were stored at 4^0 C for further studies.

Isolation from soil:

100 mg soil samples were finely powdered and stirred in one ml sterile distilled water. Serial dilutions were prepared to obtain a dilution factor 10^3. From this dilution, 100µl suspension was spread on B.R. Medium (Glucose-40g, K_2Hpo_4-1g, Kcl-0.2g, $Znso_4$-83mg, Thiamine Hcl-0.1mg, NH_4No_3-1g, $Mgso_4$-0.2g, $Feso_4$-10mg, Agar-20g and distilled water 1 litre). For one sample, three plates were used and these plates were incubated at 28^0 C for 4 days in an incubator. Typical *S. rolfsii* colonies were isolated and sub cultured on a PDA medium. This culture was incubated for 20 days for sclerotia formation and the isolates were further purified by growing single sclerotium from each colony on Potato dextrose agar (PDA) slant and maintained at 4^0 C for further studies.

All these isolates were collected in three separate surveys *i.e.* first survey 17th, 18th April 2008, second survey on 19th July 2008, and third survey on 8th October 2008. The details of all these isolates are given in Table1.

Morphological studies:

Colony morphology, colony diameter, number of sclerotia per plate, size and colour of sclerotia were evaluated on PDA. This was done by inoculating three PDA plates (90 x 15 mm) with a 5 mm diameter mycelial disc taken from the margin of an actively growing colony (3 days old) of each isolate. The inoculated plates were incubated in an incubator at 28 ± 1^0 C. The colony diameter was measured daily till 7 days. The number of sclerotia per colony was counted after 20 days of incubation. Diameter of 50 sclerotia from each plate was measured. The data from three replicated plates were averaged.

Virulence test of *S. rolfsii* isolates:

Total 59 isolate of *S. rolfsii* were screened to study most virulent strains. This was done in earthen pots. The pots were filled with 10 kg autoclaved black soil and this soil in each pot was infested with 100g inoculum of each isolate multiplied on sorghum seeds. Five pots were maintained for each isolate and five pots were served as control (without inoculum of *S. rolfsii*). In these pots, 10 seeds of GG-20 cultivar were sown and observations were recorded on seedling mortality and per cent incidence of stem rot by *S. rolfsii*.

Mycelial compatibility:

Fifteen ml of PDA medium was poured in 90 mm Petridishes (three for each isolate). These Petridishes were inoculated with 5 mm disc separated from Periphery of the actively growing 5 days old culture of each isolate. These discs were then placed opposite to each other near the periphery of Petridishes. Four isolates were usually paired on one Petridish. The Petridishes were incubated at 28^0 C in an incubator. Observations were recorded on antagonism, formation of inhibition zone from five days till 30 days after incubation.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been presented under following heads:

Morphological studies:

All the 59 isolates of *S. rolfsii* varied in all of the test parameters. Out of 59 isolates, colonies of 35 isolates were fluffy, whereas 24 were dense (compact). The growth rate of the isolates varied significantly. Thirty five isolates were the fastest growing (37 mm/d). While 24 isolates were slow growing (20 to 32 mm/d). Production of sclerotia among the isolates varied drastically. Twelve of the isolates produced more (550 to 670 sclerotia/plate) but smaller in size, while nine other isolates produced fewer (130 to 260 sclerotia/plate) which were large in size. The average size of sclerotia of most of the isolates varied from 2.1 to 2.5 mm in diameter. The colour of sclerotia was dark brown, reddish brown and light brown (Table 2).

Table 1 : Details of isolates of *S. rolfsii* Sacc. collected from different groundnut growing areas of Maharashtra and Gujarat States causing stem rot of groundnut

Sr. No.	Accession no. of isolates	Locations	Type of sample for isolation	Date of collection
1.	NRCG-SR-01	Latur (Maharashtra State)	Diseased stem	17.04.08
2.	NRCG-SR-02	Ausaa (Maharashtra State)	Soil	17.04.08
3.	NRCG-SR-03	Nilanga (Maharashtra State)	Diseased stem	17.04.08
4.	NRCG-SR-04	Latur (Maharashtra State)	Diseased stem	17.04.08
5.	NRCG-SR-05	Udgir (Maharashtra State)	Soil	17.04.08
6.	NRCG-SR-06	Ahemadpur (Maharashtra State)	Soil	17.04.08
7.	NRCG-SR-07	Udgir (Maharashtra State)	Soil	17.04.08
8.	NRCG-SR-08	Solapur (Maharashtra State)	Soil	17.04.08
9.	NRCG-SR-09	Belati (Maharashtra State)	Diseased stem	17.04.08
10.	NRCG-SR-10	Sangela (Maharashtra State)	Diseased stem	17.04.08
11.	NRCG-SR-11	Kandhar (Maharashtra State)	Diseased stem	17.04.08
12.	NRCG-SR-12	Bhokar (Maharashtra State)	Soil	17.04.08
13.	NRCG-SR-13	Loha (Maharashtra State)	Diseased stem	17.04.08
14.	NRCG-SR-14	Nanded (Maharashtra State)	Soil	17.04.08
15.	NRCG-SR-15	Savrkhed (Maharashtra State)	Soil	18.04.08
16.	NRCG-SR-16	Kevla (Maharashtra State)	Diseased stem	18.04.08
17.	NRCG-SR-17	Kushnoor (Maharashtra State)	Diseased stem	18.04.08
18.	NRCG-SR-18	Biloli (Maharashtra State)	Diseased stem	18.04.08
19.	NRCG-SR-19	Kotgil (Maharashtra State)	Diseased stem	18.04.08
20.	NRCG-SR-20	Karkheli (Maharashtra State)	Soil	18.04.08
21.	NRCG-SR-21	Junagadh (Gujarat State)	Diseased stem	19.07.08
22.	NRCG-SR-22	Junagadh (Gujarat State)	Diseased stem	19.07.08
23.	NRCG-SR-23	Junagadh (Gujarat State)	Soil	19.07.08
24.	NRCG-SR-24	Visavdar (Gujarat State)	Diseased stem	19.07.08
25.	NRCG-SR-25	Talala (Gujarat State)	Diseased stem	19.07.08
26.	NRCG-SR-26	Chitravad (Gujarat State)	Soil	19.07.08
27.	NRCG-SR-27	Somnath (Gujarat State)	Soil	19.07.08
28.	NRCG-SR-28	Veraval (Gujarat State)	Diseased stem	19.07.08
29.	NRCG-SR-29	Keshod (Gujarat State)	Diseased stem	19.07.08
30.	NRCG-SR-30	Talala (Gujarat State)	Soil	19.07.08
31.	NRCG-SR-31	Chitravad (Gujarat State)	Diseased stem	19.07.08
32.	NRCG-SR-32	Manvad (Gujarat State)	Diseased stem	19.07.08
33.	NRCG-SR-33	Prempara (Gujarat State)	Diseased stem	19.07.08
34.	NRCG-SR-34	Satadar (Gujarat State)	Diseased stem	19.07.08
35.	NRCG-SR-35	Borvav (Gujarat State)	Soil	19.07.08
36.	NRCG-SR-36	Gadu (Gujarat State)	Diseased stem	19.07.08
37.	NRCG-SR-37	Maliya (Gujarat State)	Soil	19.07.08
38.	NRCG-SR-38	Monia (Gujarat State)	Soil	19.07.08
39.	NRCG-SR-39	Junagadh (Gujarat State)	Diseased stem	19.07.08
40.	NRCG-SR-40	Borvav (Gujarat State)	Diseased stem	19.07.08
41.	NRCG-SR-41	Satadar (Gujarat State)	Soil	19.07.08
42.	NRCG-SR-42	Maliya (Gujarat State)	Diseased stem	19.07.08
43.	NRCG-SR-43	Monia (Gujarat State)	Diseased stem	19.07.08
44.	NRCG-SR-44	Veraval (Gujarat State)	Soil	19.07.08
45.	NRCG-SR-45	Borvav (Gujarat State)	Diseased stem	19.07.08
46.	NRCG-SR-46	Chitravad (Gujarat State)	Diseased stem	19.07.08
47.	NRCG-SR-47	Somnath (Gujarat State)	Soil	19.07.08
48.	NRCG-SR-48	Latur (Maharashtra State)	Diseased stem	08.10.08
49.	NRCG-SR-49	Udgir (Maharashtra State)	Diseased stem	08.10.08
50.	NRCG-SR-50	Shirur (Maharashtra State)	Diseased stem	08.10.08
51.	NRCG-SR-51	Wadhona (Maharashtra State)	Diseased stem	08.10.08
52.	NRCG-SR-52	Balsangi (Maharashtra State)	Diseased stem	08.10.08

Contd. Table 1.

Table 1 contd…

53.	NRCG-SR-53	Hadolti (Maharashtra State)	Diseased stem	08.10.08
54.	NRCG-SR-54	Umrga (Maharashtra State)	Soil	08.10.08
55.	NRCG-SR-55	Jalkot (Maharashtra State)	Soil	08.10.08
56.	NRCG-SR-56	Jalkot (Maharashtra State)	Diseased stem	08.10.08
57.	NRCG-SR-57	Latur (Maharashtra State)	Soil	08.10.08
58.	NRCG-SR-58	Udgir (Maharashtra State)	Soil	08.10.08
59.	NRCG-SR-59	Udgir (Maharashtra State)	Diseased stem	08.10.08

Table 2 : Morphological variation in different isolates of *S. rolfsii* after 20 days of inoculation

Sr. No.	Accession no. of isolates	Colony diameter (mm) *	Colony type	No. of sclerotia*	Sclerotial size (mm)*	Sclerotial colour
1.	NRCG-SR-01	77.67	Dense	221	2.2	DB
2.	NRCG-SR-02	88.00	Fluffy	568	1.0	LB
3.	NRCG-SR-03	87.33	Fluffy	576	1.2	DB
4.	NRCG-SR-04	89.00	Fluffy	612	1.2	LB
5.	NRCG-SR-05	85.00	Fluffy	363	1.1	RB
6.	NRCG-SR-06	68.33	Dense	260	2.2	DB
7.	NRCG-SR-07	79.00	Dense	366	1.4	DB
8.	NRCG-SR-08	90.00	Fluffy	278	2.1	LB
9.	NRCG-SR-09	85.67	Fluffy	448	1.4	LB
10.	NRCG-SR-10	82.00	Fluffy	503	1.0	DB
11.	NRCG-SR-11	75.33	Fluffy	543	1.1	RB
12.	NRCG-SR-12	61.00	Dense	286	2.1	DB
13.	NRCG-SR-13	86.00	Fluffy	457	0.9	LB
14.	NRCG-SR-14	90.00	Fluffy	368	1.6	LB
15.	NRCG-SR-15	76.33	Fluffy	437	1.4	DB
16.	NRCG-SR-16	77.67	Fluffy	543	1.4	DB
17.	NRCG-SR-17	90.00	Fluffy	408	1.5	DB
18.	NRCG-SR-18	90.00	Fluffy	565	1.2	RB
19.	NRCG-SR-19	90.00	Fluffy	398	1.1	DB
20.	NRCG-SR-20	61.33	Dense	274	2.5	DB
21.	NRCG-SR-21	85.67	Fluffy	396	1.2	DB
22.	NRCG-SR-22	83.33	Fluffy	404	1.2	LB
23.	NRCG-SR-23	78.67	Dense	336	1.8	LB
24.	NRCG-SR-24	64.67	Dense	149	2.2	DB
25.	NRCG-SR-25	72.00	Fluffy	473	1.1	DB
26.	NRCG-SR-26	82.67	Fluffy	523	1.0	LB
27.	NRCG-SR-27	75.67	Dense	168	2.1	LB
28.	NRCG-SR-28	84.00	Fluffy	337	1.1	LB
29.	NRCG-SR-29	90.00	Fluffy	455	1.2	RB
30.	NRCG-SR-30	90.00	Fluffy	509	0.5	DB
31.	NRCG-SR-31	88.67	Fluffy	487	0.8	RB
32.	NRCG-SR-32	80.33	Fluffy	392	1.2	DB
33.	NRCG-SR-33	87.33	Fluffy	489	1.5	DB
34.	NRCG-SR-34	68.00	Dense	317	1.9	DB
35.	NRCG-SR-35	76.00	Fluffy	464	1.2	RB
36.	NRCG-SR-36	84.00	Fluffy	528	1.4	DB
37.	NRCG-SR-37	63.33	Dense	344	1.8	DB
38.	NRCG-SR-38	74.00	Fluffy	354	1.0	LB
39.	NRCG-SR-39	80.33	Fluffy	497	1.4	RB

Contd.... Table 2

Table 2 contd…

40.	NRCG-SR-40	90.00	Fluffy	502	0.9	RB
41.	NRCG-SR-41	90.00	Fluffy	487	1.0	DB
42.	NRCG-SR-42	39.00	Dense	378	1.8	DB
43.	NRCG-SR-43	57.00	Dense	144	2.1	LB
44.	NRCG-SR-44	63.33	Dense	335	1.9	DB
45.	NRCG-SR-45	53.67	Dense	314	2.0	DB
46.	NRCG-SR-46	57.33	Dense	378	1.8	DB
47.	NRCG-SR-47	57.00	Dense	312	1.6	RB
48.	NRCG-SR-48	68.00	Dense	314	1.9	DB
49.	NRCG-SR-49	61.33	Dense	187	2.1	DB
50.	NRCG-SR-50	64.33	Dense	387	1.8	RB
51.	NRCG-SR-51	68.33	Dense	356	2.1	LB
52.	NRCG-SR-52	85.67	Fluffy	452	0.9	LB
53.	NRCG-SR-53	90.00	Fluffy	469	1.0	RB
54.	NRCG-SR-54	65.67	Dense	339	1.9	RB
55.	NRCG-SR-55	60.00	Dense	387	2.0	LB
56.	NRCG-SR-56	55.67	Dense	314	1.9	DB
57.	NRCG-SR-57	71.33	Fluffy	358	1.1	DB
58.	NRCG-SR-58	90.00	Fluffy	514	0.9	DB
59.	NRCG-SR-59	90.00	Dense	381	2.0	LB

Note: * Mean of three replications, DS-Diseased Stem, DB-Dark Brown, LB- Light Brown, RB- Reddish Brown

Table 3 : Virulancy variation in different isolates of *S. rolfsii in* pot

Sr. No.	Accession no. of isolates.	% Germination*	% Incidence of stem rot*
1.	NRCG-SR-01	86.7	10.0
2.	NRCG-SR-02	80.0	13.3
3.	NRCG-SR-03	73.3	56.7
4.	NRCG-SR-04	93.3	6.7
5.	NRCG-SR-05	90.0	13.3
6.	NRCG-SR-06	73.3	63.3
7.	NRCG-SR-07	80.0	73.3
8.	NRCG-SR-08	93.3	6.7
9.	NRCG-SR-09	86.7	70.0
10.	NRCG-SR-10	93.3	3.3
11.	NRCG-SR-11	100.0	13.3
12.	NRCG-SR-12	83.3	13.3
13.	NRCG-SR-13	86.7	16.7
14.	NRCG-SR-14	100.0	6.7
15.	NRCG-SR-15	83.3	20.0
16.	NRCG-SR-16	90.0	6.7
17.	NRCG-SR-17	86.7	73.3
18.	NRCG-SR-18	90.0	76.7
19.	NRCG-SR-19	83.3	6.7
20.	NRCG-SR-20	76.7	0.0
21.	NRCG-SR-21	96.7	20.0
22.	NRCG-SR-22	83.3	10.0
23.	NRCG-SR-23	83.3	10.0
24.	NRCG-SR-24	100.0	6.7
25.	NRCG-SR-25	93.3	6.7
26.	NRCG-SR-26	70.0	76.7
27.	NRCG-SR-27	76.7	80.0
28.	NRCG-SR-28	83.3	13.3
29.	NRCG-SR-29	83.3	6.7

Contd…. Table 3

Table 3 contd….

30.	NRCG-SR-30	96.7	10.0
31.	NRCG-SR-31	90.0	10.0
32.	NRCG-SR-32	100.0	23.3
33.	NRCG-SR-33	70.0	13.3
34.	NRCG-SR-34	93.3	13.3
35.	NRCG-SR-35	93.3	13.3
36.	NRCG-SR-36	100.0	13.3
37.	NRCG-SR-37	93.3	10.0
38.	NRCG-SR-38	96.7	16.7
39.	NRCG-SR-39	100.0	10.0
40.	NRCG-SR-40	86.7	10.0
41.	NRCG-SR-41	96.7	6.7
42.	NRCG-SR-42	100.0	13.3
43.	NRCG-SR-43	96.7	13.3
44.	NRCG-SR-44	86.7	13.3
45.	NRCG-SR-45	100.0	23.3
46.	NRCG-SR-46	96.7	23.3
47.	NRCG-SR-47	90.0	13.3
48.	NRCG-SR-48	86.7	20.0
49.	NRCG-SR-49	100.0	16.7
50.	NRCG-SR-50	76.7	13.3
51.	NRCG-SR-51	100.0	23.3
52.	NRCG-SR-52	96.7	16.7
53.	NRCG-SR-53	93.3	23.3
54.	NRCG-SR-54	100.0	13.3
55.	NRCG-SR-55	96.7	20.0
56.	NRCG-SR-56	93.3	10.0
57.	NRCG-SR-57	80.0	70.0
58.	NRCG-SR-58	90.0	20.0
59.	NRCG-SR-59	96.7	16.7
Control		100	0.0

*Average of five replications

Virulence test of *S. rolfsii* isolates:

Out of 59 isolates only nine isolates were found to be highly virulent causing more than 60% mortality of plants due to stem rot. The details are shown in Table 3.

Mycelial compatibility:

There were 400 pairings of the 59 isolates and out of all, 129 combinations showed a compatible reaction (32.3% of all the combinations). The remaining 271 combinations were incompatible where mycelia of the two isolates interacted at the zone of interaction. The incompatible combinations showed antagonistic reactions with each other, forming a thin band of living or dead mycelia. In some combinations, after 20 days of incubation, hyphae of one isolate began to turn black and subsequently lysed. In few isolates the interacting zone varied from 5 mm to 9 mm. The high rate of antagonistic reactions in the mycelial compatibility test further showed the extent of the diversity among these isolates of *S. rolfsii*. The death of mycelia at the interaction zone has been attributed to the heterokaryotic condition of the nuclei (Punja, 1985), but the involvement of toxin cannot also be ruled out (Punja, 1985).

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